

Structure of intercellular airspace in the lamina of floating leaves of *Nymphaea candida* C. Presl (Nymphaeaceae): bizarre combination of homobaric and heterobaric types

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Summary: The whole mesophyll of floating leaves of *Nymphaea candida* is chambered. Both palisade and spongy chlorenchymata consist of 1–2-layered septa between substomatal chambers and huge airspaces, respectively. The septa of the spongy chlorenchyma are imperforate and make this tissue mass heterobaric in the absence of bundle-sheath extensions of medium-sized veins. The septa of the palisade chlorenchyma are increasingly imperforated near the stomata, but they become increasingly perforated inwards and pass into a loose network of the collecting cells (Sammelzellen) to make the palisade chlorenchyma mass homobaric. The palisade chlorenchyma of chambered type is unlikely to need lateral gas diffusion through the mesophyll to maintain its photosynthesis.

Keywords: heterobaric leaf, homobaric leaf, chambered mesophyll, substomatal chambers, floating leaf, *Nymphaea candida*

Laminas of floating leaves of representatives of Nymphaeaceae are usually described as typically dorsiventral having a thick, multilayered adaxial palisade chlorenchyma and an abaxial spongy one (KAUL 1976; CHEN et al. 1987; CHEN & ZHANG 1992; CATIAN & SCREMIN-DIAS 2013). The spongy chlorenchyma is peculiar in all but *Brasenia* members of this family. It consists of non-lobed tightly arranged cells which constitute single-layered imperforate septa, the latter separating huge closed air chambers (KAUL 1976; CHEN et al. 1987; CHEN & ZHANG 1992).

The palisade chlorenchyma looks quite ordinary against the background of such a peculiar spongy counterpart. It is described as multilayered tissue of tightly packed, quite long cells (KAUL 1976; CHEN et al. 1987; CHEN & ZHANG 1992; CATIAN & SCREMIN-DIAS 2013). It is permeated by long narrow substomatal chambers which open into the adaxialmost air chambers of the spongy chlorenchyma (KAUL 1976; CATIAN & SCREMIN-DIAS 2013).

Dense arrangement of the palisade cells looks usual in hypostomatic leaves, but not in the epistomatic leaves of Nymphaeaceae, because it does not fit the efficient gas exchange of the palisade chlorenchyma of the latter. The stomata are patchy in the adaxial epidermis to cause obligatory lateral gas diffusion through the mesophyll (GUTTENBERG 1963; PARKHURST 1994). The palisade chlorenchyma of tightly arranged cells is too resistant to lateral gas diffusion to confine it to the spongy one (TERASHIMA 1992; PARKHURST 1994; EVANS & LORETO 2000; PIERUSCHKA et al. 2005; MORISON & LAWSON 2007). In laminas of floating leaves of Nymphaeaceae, such a mode of lateral gas allocation in the mesophyll would compulsorily involve direct cross-leaf diffusion of CO₂ through substomatal chambers, its subsequent lateral diffusion in the air chambers of spongy chlorenchyma and complete recurrent cross-leaf diffusion through narrowest intercellular air spaces in the palisade tissue (Fig. 1). Photosynthetically released O₂ would run the same pathway in the opposite direction. Such diffusion would almost double the distance gas has to

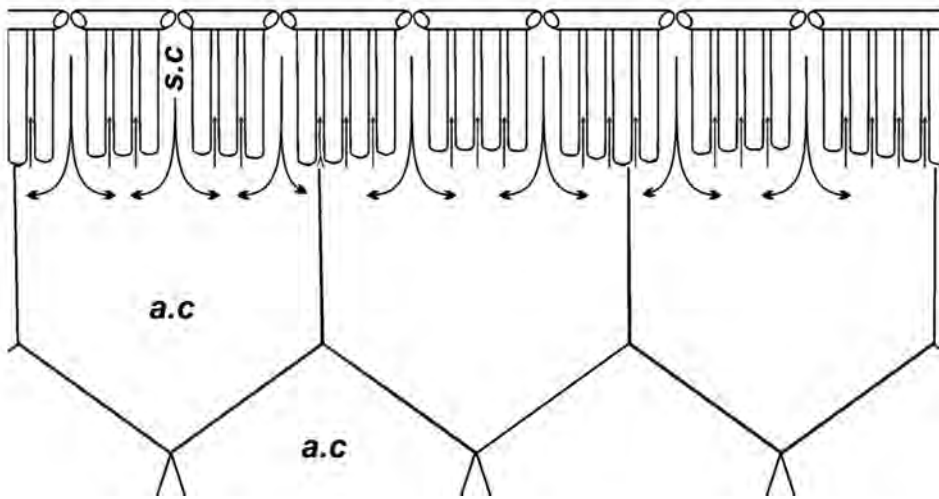


Figure 1. Conjectural diffusion of gases in the mesophyll of a floating leaf of Nymphaeaceae. *a.c* – air chamber of the spongy chlorenchyma; *s.c* – substomatal air chamber.

run. It would be equivalent to unidirectional cross-leaf diffusion through a twice thicker palisade chlorenchyma.

Thickness of the leaf palisade chlorenchyma seems to have never been summarized. Data are scanty and scattered in literature. A screening of the publications has shown that the thickness of the palisade chlorenchyma of dorsiventral leaves never exceeds 210–220 μm (MEYER 1962; NAPP-ZINN 1973; KIMMERER & POTTER 1987; ROTH 1990; JAMES & BELL 1995, 2000; HUNTER 1997; GRATANI et al. 2000, 2003; MEDIIVILLA et al. 2001). It is mostly even less than 100 μm thick (POPLAVSKAYA 1948; RUDALL 1980¹; ROTH 1990; ASHTON & BERLYN 1992; CHAZDON & KAUFMAN 1993; SHI & CAI 2008; PANDITHARATHNA et al. 2008; MATOS et al. 2009; FINI et al. 2010; HERNANDEZ et al. 2016²). The palisade chlorenchyma of floating leaves is 120–150 μm thick in *Nymphaea candida* C. Presl (TIMONIN & NOTOV 1993; BARYKINA & CHUBATOVA 2005), approximately 110 μm in *Nymphaea amazonum* Mart. & Zucc. (CATIAN & SCREMINDIAS 2013) and 170 μm in *Nuphar* sp. (MEYER 1962). If doubled, the thickness of the palisade chlorenchyma in Nymphaeaceae would thus highly exceed that in most dorsiventral leaves. It would be comparable to the total palisade chlorenchyma thicknesses in equifacial leaves as evidenced by RUDALL (1980), TIMONIN & NOTOV (1993), BARYKINA & CHUBATOVA (2005) and seemingly by EDWARDS et al. (2000). However, equifacial leaves are amphistomatic (NAPP-ZINN 1973). Accordingly, the cross-leaf diffusion of gases in their palisade chlorenchymata is unlikely to exceed 180–200 μm , just as in the dorsiventral leaves.

That is why the conjectural lateral gas diffusion through the palisade chlorenchyma in floating leaves of Nymphaeaceae seems to be non-functional. The efficient mode of lateral gas diffusion through the palisade chlorenchyma in floating leaves of Nymphaeaceae remains enigmatic. Therefore, the structure of the intercellular airspaces of these leaves is worth being scrutinized to elucidate this enigma.

¹ According to the bars in Figs.

² 336.5 μm and 220.9 μm are given in the Table, but the bars in photos show that the palisade chlorenchyma is about 60 μm thick or less.

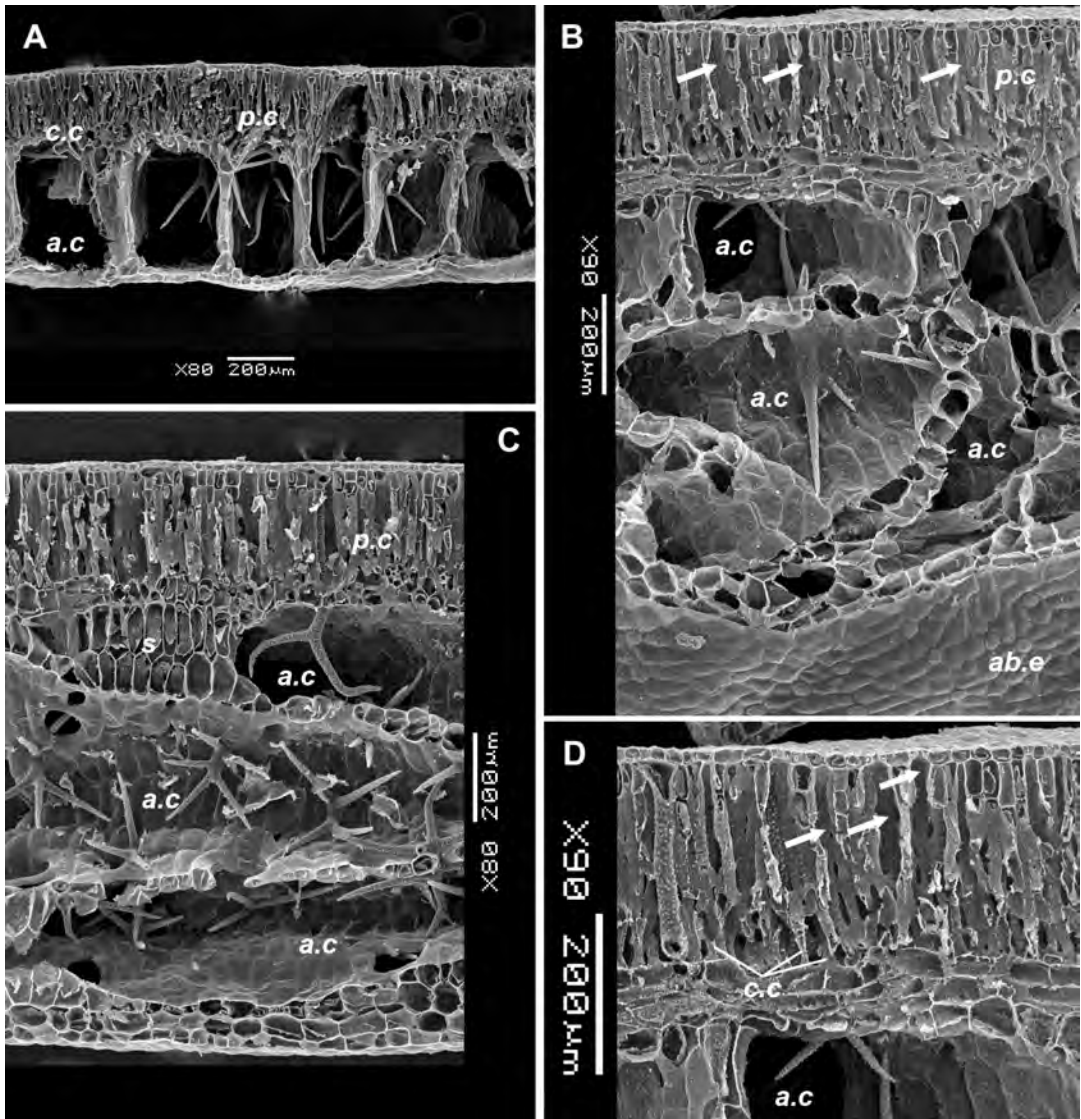


Figure 2. Transverse sections of the lamina. A – marginal part of lamina; B – central part of lamina; C – longitudinally sectioned air ducts; D – palisade chlorenchyma. *a.c* – air chamber of the spongy chlorenchyma; *ab.e* – abaxial epidermis; *c.c* – collecting cell (Sammelzelle); *p.c* – palisade chlorenchyma; *s* – sectioned septum of the air chamber; *arrows* – substomatal chambers.

Materials and methods

Laminas of floating leaves of indigenous *N. candida* were taken in the vicinity of Moscow and fixed in 70% ethyl alcohol. The fixed laminas were semi-thick sliced transversely and obliquely paradermally with hand razor. The sections were dehydrated in a series of 80%, 90%, 100% and 100% ethyl alcohol and twice 100% acetone and critical-point dried in HCP-2 Drier (Hitachi) and mounted on stubs. The sections thus prepared were coated with Pd-Au mixture in IB-3 Ion Coater (EIKO) and examined and photographed under scanning electron microscope Camscan-S2 (Cambridge Instruments). The work was carried out at Electron Microscopy Laboratory of Lomonosov Moscow State University.

Results

Huge air chambers of the spongy chlorenchyma are 1-seriate near the lamina margin (Fig. 2A) and 2- to 3-seriate in its central part (Fig. 2B). They vary from long ducts (Fig. 2C) accompanying larger veinlets to rather isodiametric intercostal cavities (Fig. 3B). The air chambers are typically isolated from each other by single-layered septa of non-lobed, tightly arranged cells of 'spongy' chlorenchyma (Fig. 2B), but various perforations sporadically occur in some septa (Fig. 2C).

The substomatal chambers are long narrow cross-leaf ducts (Fig. 2D) whose diameter slightly exceeds that of the stoma (Fig. 3D). Every chamber permeates through the palisade chlorenchyma mass (Fig. 2B, D). Some substomatal chambers merge with the air chambers of spongy chlorenchyma (Figs 2A; 3B, F), while others seem to be completely isolated from the latter (Figs 2B; 3F). The substomatal chambers are separated by septa of long palisade cells (Figs 2D; 3C). The septa are mostly 1-layered, but 2-layered ones are also present (Fig. 3C, D). The palisade cells are mostly tightly arranged to isolate neighboring substomatal chambers near the adaxial epidermis (Fig. 3D). Few small intercellular airspaces permeate the septa in the middle part of the chambers (Fig. 2D). The septa internally disintegrate into a net of collecting cells (Sammelzellen) (Figs 2D; 3E). Their intercellular airspaces unite all substomatal chambers.

Most palisade cells arranged in 1-layered septa directly contact with 2 substomatal chambers (Fig. 3C, D). Fewer cells located in 2-layered septa contact with only 1 substomatal chamber (Fig. 3C). The palisade cells that clamp three 1-layered septa contact with 3 ones (Fig. 3C).

Discussion

Specific chlorenchyma of mostly 1-layered septa between huge air chambers was determined as chambered mesophyll by KAUL (1976). Such a chambered tissue is characteristic of aquatic plants (ARBER 1920; KAUL 1976; LANDOLT et al. 1998). It is thought to enhance plant buoyancy (KAUL 1976; CATIAN & SCREMIN-DIAS 2013).

Chambered structure of the spongy chlorenchyma of Nymphaeaceae has long been known (KAUL 1976; CHEN et al. 1987; CHEN & ZHANG 1992). Surprisingly, the palisade chlorenchyma in floating leaves of *N. candida* is also chambered, but its chambers are long, narrow cross-leaf substomatal chambers which permeate the palisade tissue throughout. Every cell of this palisade chlorenchyma directly adjoins at least 1 substomatal chamber; most of them adjoin 2 chambers. Therefrom, efficient gas exchange of the palisade cells needs neither gas lateral diffusion in the palisade chlorenchyma nor gas lateral diffusion in the air chambers accompanied by the cross-leaf diffusion through narrow intercellular airspaces in the palisade chlorenchyma. Because of this, most palisade cells can be tightly arranged without harming their photosynthesis.

The substomatal chambers are highly isolated from each other by septa of palisade cells. However, they communicate through few small perforations in the septa and they are completely united by means of intercellular airspaces between collecting cells. There is a continuous compartment of the lamina intercellular airspace in the palisade chlorenchyma. Some air chambers of the spongy chlorenchyma are also united with this compartment. The air chambers of the spongy chlorenchyma are isolated from each other. Holes in their septa are irregular and scanty. They seemingly result from astrosclereids being ripped out by leaf sectioning. The air chambers are likely to be separate compartments of the lamina intercellular airspace. Therefore, the lamina intercellular airspace of floating leaves of *N. candida* is not a whole, but a set of compartments.

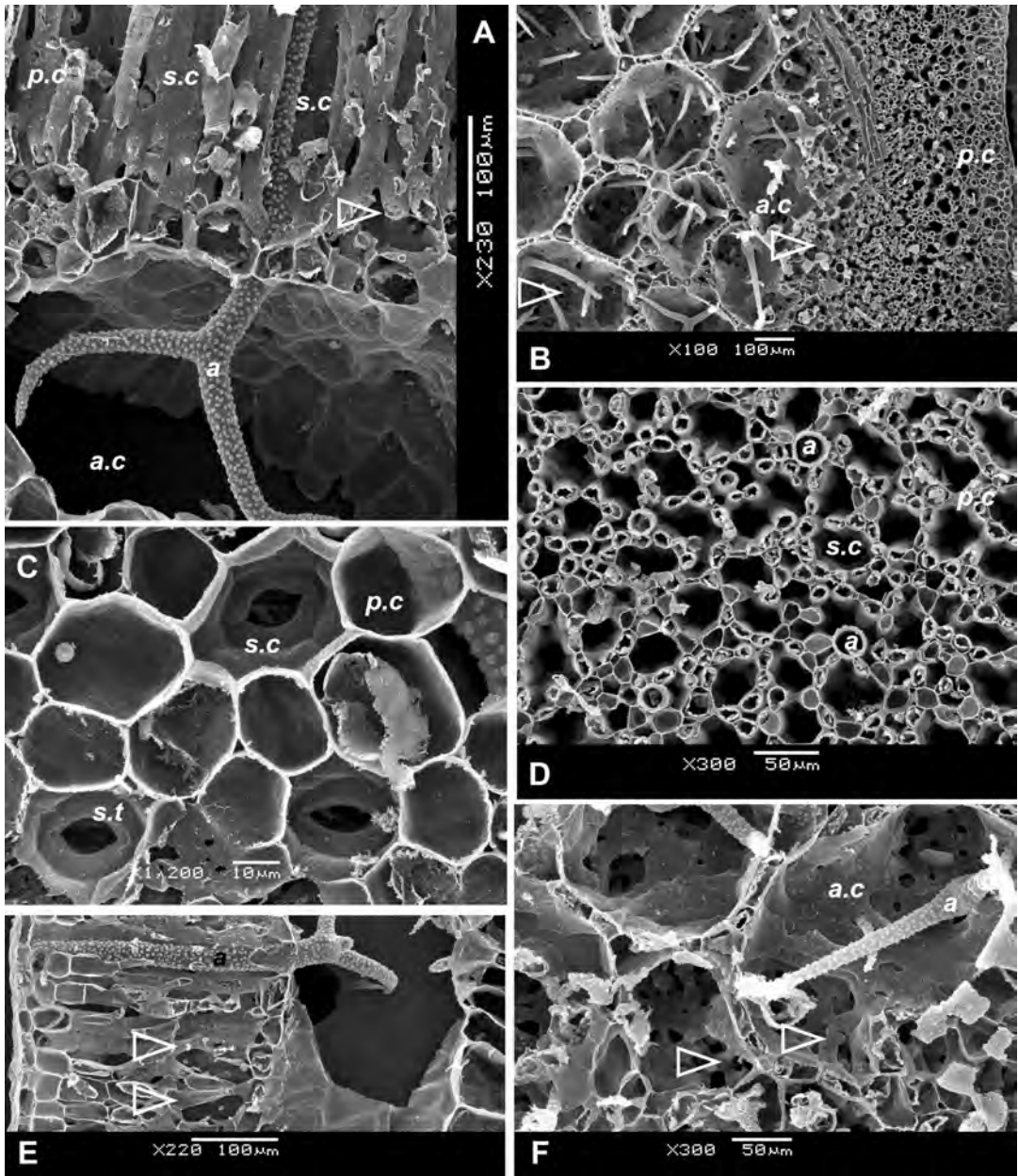


Figure 3. Transverse (A & E) and oblique paradermal (B–D & F) sections of the lamina. A – interface between palisade and spongy chlorenchymata; B – palisade and spongy chlorenchymata; C – substomatal chambers; D – palisade chlorenchyma; E & F – collecting cells (Sammelzellen). *a* – astroscleireid; *a.c* – air chamber of the spongy chlorenchyma; *p.c* – palisade chlorenchyma; *s.c* – substomatal chamber; *st* – stoma; *arrowhead* – collecting cells (Sammelzellen).

Isolated compartments of the intercellular airspace in the lamina are considered an attribute of the heterobaric leaf type (NEGER 1918). NEGER (l. c.) recognized that boundaries of intercellular airspace compartments spatially coincide with the medium-sized veins. Heterobaric leaf structure was later on invariably associated with the bundle-sheath extensions (HABERLANDT 1924; WYLIE 1952; TERASHIMA 1992; JAHNKE & PIERUSCHKA 2006; KENZO et al. 2007; MORISON et al. 2007; LEEGOOD 2008), though compartmentalization of the intercellular airspace system of the lamina

was sometimes questioned to be the principal function of the bundle-sheath extensions (BUCKLEY et al. 2011; LYNCH et al. 2012).

Nymphaea candida has no bundle-sheath extensions of medium-sized veins to compartmentalize the intercellular airspace system of the lamina. However, this system is really compartmentalized due to tight arrangement of the cells of chambered spongy chlorenchyma. If compartmentalization of the intercellular airspace system of lamina is accepted as an essence of the heterobaric leaf (NEGER 1918), the floating leaves of *N. candida* must be considered heterobaric. Scanty and occasional perforations in the septa of the spongy chlorenchyma are unlikely to negate this conclusion, because some compartments of the intercellular air cells are not absolutely isolated in leaves which look like typical heterobaric (EVANS et al. 2009).

Heterobaric structure of the floating leaf laminas in *N. candida* is due to the chambered structure of its mesophyll. Yet, there is only the chambered spongy chlorenchyma that makes the lamina heterobaric, whereas the chambered palisade chlorenchyma does not compartmentalize the intercellular airspace system. The chambered type of mesophyll thus variously combines with the heterobaric type of leaf. Correlations between chambered chlorenchymata and heterobaric leaves are worth being investigated further on.

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